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TITLE: Rapid Malaria Test for Diagnosis and Treatment

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Portland, Oregon 97201

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12a. DISTRIBUTION / AVAILABILITY STATEMENT
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12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

Malaria infects over 200 million people and is responsible for 2 million deaths annually. Flow Inc. has developed diagnostic and culture and sensitivity procedures based upon the fact that the parasite contains an enzyme lactate dehydrogenase (pLDH) (US patent 5124141) Flow Inc. recently increased the sensitivity to 50-100 parasites/ul with monoclonal antibodies. These newly formatted wet (immunocapture), and dry (immuno-chromatographic) assays, can differentiate *Plasmodium falciparum from other Plasmodium sp.* that infect humans; and appears to be able to monitor the effectiveness of *in vivo* and *in vitro* drug therapy.

The development of such a rapid simple to perform, easy to interpret, diagnostic, and *in vitro* and *in vivo* therapeutic assays for the malaria parasite has application for diagnosis and therapeutic monitoring in the developing world; for US troops stationed in these areas of the world ; for epidemiological surveillance; and to monitor the effectiveness of new antimalarials *in vitro* and *in vivo.*

14. SUBJECT TERMS	15. NUMBER OF PAGES
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FOREWORD

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____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

MICHAEL T. MAKLER, M.D. **4/22/97**

PI - Signature Date

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I SPECIFIC AIMS

Flow Inc. intends to demonstrate during the 6 month SBIR I period the feasibility of using both its immunocapture (wet) and immunochromatographic (dry) assays for rapid, low cost, simple, accurate, quantitative, diagnosis and speciation of the human malaria parasites and to demonstrate the application of these assays to vaccine trials, blood product monitoring and therapeutic surveillance.

II PROGRESS REPORTS (1-6)

A. ADMINISTRATION

The technical staff during this 6 months included Michael T. Makler(PI), Robert C. Piper, Jean Williams and Junita Ries, Diane Schacht, and Laura Wentworth. Administrative staff include accountants at Kent and Snow (Selma Kotchik) and Sharon Makler at Flow Inc.

B. RESEARCH PROGRESS

REPORT 1

During this period Dr. Makler spent time preparing preliminary reports for evaluation of the OptiMAL® by the FDA, and one week with a visitor from the Army of Korea. Dr. Lim brought over 300 cases of *vivax* malaria to Flow's Laboratory in Portland, OR to be evaluated. During the remainder of the month Dr. Makler, spent time evaluating data obtained from Paris (Pasteur and Hopital Bichard 8/96) and the UK(HTD), and from two field studies conducted with the OptiMAL® reagents in The Gambia(11/96) , by Angela Hunt-Cooke of the HTD, London and from Honduras by Rob Piper(Flow Inc. 11/96) with Dr. Martin and Dr Quitana (AFIP). Analysis of the data from Europe and from both field studies is in progress. During the first week of December Dr. Makler and Dr. Piper were in Baltimore, during the second week of December, Professor Le Bras (Paris) visited Flow. Together, both Dr. Makler and Dr. Lebras re-ran and re-analyzed a large amount of anonymous patient samples and data obtained from an anti-malarial study performed in Paris in August 1996. This reevaluation was done utilizing both the wet immunocapture pLDH and dry OptiMAL® assays. The results are being prepared for publication.

REPORT 2

During this period Dr. Makler continued to interact with the FDA in hopes of achieving FDA status for the technologies. The current opinion is that a PMA is required. A large number of studies were performedon gold conjugate that was made in-house to determine whether the sensitivity of the dry assay could be further increased. The maximum sensitivity with frozen whole blood samples is approximately 100 parasites/ul. Evaluation of the new gold conjugates with a different set of buffers, of varying protein composition was completed. This confirms the need for a minimal concentration of casein. Additional time was spent analyzing Parisian data (Pasteur and Hopital Bichard 8/96) and data from the UK(HTD). Continued microscopic examination of 465 slides from The Gambi (11/96) , by Angela Hunt-Cooke of the HTD, London and from 400 blood films from the Honduras field study by Rob Piper(Flow Inc. 11/96) with Dr. Martin and Dr Quitana (AFIP)continues.

During the last 2weeks of December more effort was made to improve the gold colloid, and made to determine whether latex beads would increase assay sensitivity. Results are

promising. Dr. Makler introduced a method to enhance the gold signal. Initial experiments showed that there was in fact real enhancement , but noise level of the uninfected control are also present. This suggests that additional blocking steps need to be introduced.

REPORT 3

During this period Dr. Makler received a report from the FDA that precludes Flow from obtaining orphan status for the OptiMAL ® technology. The FDA has stated that a PMA is required. This letter was forwarded to Colonel Milhous for further action. Studies continue on the evaluation of the assay in developing countries. Approximately 200 blood samples were received from Honduras. These were examined by the OptiMAL® assay, the lcpLDH assay and thin and thick blood smear. There was excellent correlation and sensitivity and specificity studies were well above 90%. Almost 100% of the 83 positive smears were diagnosed as *Plasmodium vivax* in Honduras; whereas the OptiMAL® and lcpLDH demonstrated that 15 of these were *P. falciparum* . This was confirmed by microscopy in Oregon. This disparity is significant as all persons diagnosed with malaria (*P.vivax*) in Honduras are being treated with chloroquine!

The microscopic reading of the Gambian samples, 409 thin and thick smears were completed and compared to the OptiMAL® assay . The Excel file-, the initial analysis is included in the report (NOT FINAL REPORT) . Further analysis is **ongoing.**

The rare reagents, the mabs used in both pLDH assays, are being produced at a facility with GMP. This is in anticipation of producing GMP kits that will be used for field studies in the **phase II** part of this research contract. Preliminary material received from the GMP facility has been tested in both dry and wet assays and has been demonstrated to identical functional activity as the original products produced from the hybridomas.

Corporate activity is ongoing, a GMP manufacture has been defined, and contracts are under way. This manufacturer will be responsible for the production of all the kits for the future field studies. These studies are anticipated to begin in **phase II.**

REPORT 4

During this period Flow Inc. has completed the analysis of two field studies , examined and produced strips in a GMP facility, completed work on the preparation to produce 100 kits of 100 strips each for distribution to the US AID/WHO for field evaluation in **Africa and written a large series of reports.**An insert for the OptiMAL® kit is appended.

Flow Inc. has also performed both the OptiMAL®, lcpLDH and microscopic analysis on a second Honduras study carried out in collaboration with Dr. Palmer and Dr. Ager of the Department of Infectious Disease, U. of Miami. The results of this study are reported in the table below. A manuscript is in preparation. Briefly 189 patients with clinical symptoms were examined in malaria clinics in Honduras . A portion of their blood samples were dispatched to the U. of Miami where the samples were further analyzed by both microscopy and the OptiMAL® assay. An additional numbered aliguot of whole blood was sent to Flow Inc. Flow Inc. had no idea of persons identity. This sample was analyzed at Flow Inc. by the OptiMal® assay and the lcpLDH assay. A small number (30) thin and thick smears were made. Correlation between the OptiMAL® and lcpLDH assays is 100%. The results of the study indicate that about 50% of the clinic patients had malaria. Approximately 85% had *P. vivax* and 15% *P. falciparum.* It should be noted that this is a 10 fold increase in the incidence of *P.falciparum* from 1993(WER #4

1996). The data also revealed that the *P. falciparum* is clustered to a few houses in one or two villages located on the northern most shore of Honduras.

What was particularly interesting about this field study , is the fact that all the malaria diagnosed and treated at the Hondurian clinics was speciated as *P.vivax*. Wheras the US readers confirmed P. falciparum by microscopy as well as by the OptiMAL®, and further confirmed these cases of *P. falciparum* with the HRP2 antigen test.

The microscopic reading of the Gambian samples by three independent readers, that is 409 thin and thick smears, has been completed and compared to the OptiMAL® assay . A previous report showed some of this data. The Gambian study described in part in a table below shows the discrepant results. The two tables below this table compare the results from the microscopists with the OptiMAL® assay. It should be noted that most of the samples were read by the Gambian reader as *P. falciparum*. They did not read *P. malariae* or *P. ovale*. This was not the case by the UK and USA readers, who commented on different species.

Flow Inc. has received two of its rare reagents-the mabs used in both the above pLDH assays. These mabs are being produced at a facility with GMP and which is FDA approved. Flow Inc. has committed to this in order to use these reagents in test kits manufactured in a GMP facility in the **phase II** part of this research contract(if awarded) in order to achieve FDA approval. Three of the mabs received have been tested in both the dry (OptiMAL®) and the wet (IcpLDH) assays. It has been demonstrated that these mabs have identical functional activity to the original preparations produced from hybridomas.

Corporate activity at Flow Inc. is ongoing. Several visits to Flow Inc. have occurred during this period. The corporate associate has confirmed the validity of the OptiMAL® assays. A GMP manufacture has been defined. An initial small number of Flow Inc. strips are being manufactured at this facility. Flow Inc. will test these to see if they meet QC requirements. If this is the case, then this GMP manufacturer may be designated for the production of kits for future field studies. These field studies are anticipated if a **phase II** award is received.

REPORT 5

The period from 3/15-4/15 was concerned with the production of 10,000 OptiMAL® strips for distribution to USAID/WHO and the DOD. This included the formatting of 100 kits of 100 dip sticks/kit. The strips were stripped at Flow Inc. and laminated (a new process) at Flow Inc. They were then placed in dessicated containers (50 sticks). The kit contained 100 plastic pipettes that aspirate and deliver 10ul , 200 wells to perform the tests, graphic and written instructions.
This period was also concerned with evaluating a GMP manufacturing site in Southern California and of meeting several times with potential corporate partners. This was very time consuming.
During this period we discovered another process to stabilize the gold conjugate. This involves lyophilization with a few additives.
Several graphic productions were completed. Some of these were included in the kit.
Dr. Makler spent 2 weeks in Europe at the HTD, Pasteur and at Hospital Bichat. Several manuscripts were reviewed, and the field study in the Gambia was reviewed. At the Pasteur , a new modification of the IcpLDH was perfected. This technique is able to detect 1prbc/10e9RBC. We completed a field study in Honduras with Dr.Palmer and Dr. Ager. With our recombinant we established a series of standards for evaluation of kits etc.

FINAL MONTH

REPORT 6 (FINAL)

This month was involved in completing manuscripts for submission for publication. Writng and submitting a: phase II proposal; a BAA, proposal; and an FDA protocol. Flow Inc. also, continued studies on: evaluating the stability of its reagents; quality controlled studies on reagents from sub contractors; providing kits to requestors; compared the method to the hrp 2 assay; and commenced studies to adapt the OptiMAL® dip stick method to a single use stick that would be able to be carried as a single enclosed unit; testing polyclonal reagents for their ability to detect *P. ovalae* and *P. malariae.*

In summary , Flow Inc. has proven in laboratory and field studies that its wet and dry pLDH assays are able to diagnose, speciate and monitor antimalarial therapy. Thus Flow Inc. has successfully completed all the objectives of this phase I project.

III PROBLEM AREAS

No specific problems in research design or protocols have been revealed to date. The major use for the technology is the third world. Here funding depends upon institutions such as the DOD, US AID or WHO. All these parties want to discount the technology to a point there is no profit margin. This is a major problem in finding a commercial partner!

IV FUTURE WORK

Flow Inc. has submitted a **phase II** proposal defining in detail the work it intends if this is awarded . This includes 3 objectives:

To improve the sensitivity and stability of the pLDH assay
To improve the specificity of the assay
To perfect an OptiMAL® quantitative strip.

As mentioned, a BAA proposal was also submitted. This proposal is intended to acquire the funds necessary to achieve FDA approval for the OptiMAL® diagnostic assay.

Commercialization of Flow Inc.'s products remains a primary concern and this activity is being pursued aggressively. This commercialization requires a skilled business administrator and negotiator.

Flow Inc. has received a significant number of orders for the OptiMAL® sticks, their Malstat™ reagent and the lcpLDH coated plates

Michael T. Makler, MD.

Bibliography:

Many manuscripts are in preparation:
Palmer, Ager et al Malaria in Honduras-submitted
Palmer, Ager et al, OptiMAL® dip stick diagnosis in Honduras
Piper et al, Diagnostic methods for P. falciparum-submitted Lancet
Chiodini et al, Gambian Study and OptiMAL®
Vanderjagt et al, Biochemistry of pLDH-submitted
Lim et al, OptiMAL® in Korea
Quitana et al, OptiMAL/PCR in Honduras

DEPARTMENT OF THE ARMY
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
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